

Probing the Mechanical Unzipping of DNA

N. K. Voulgarakis, A. Redondo, A. R. Bishop, and K. Ø. Rasmussen

Theoretical Division and Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, USA

(Received 20 December 2005; published 21 June 2006)

A study of the micromechanical unzipping of DNA in the framework of the Peyrard-Bishop-Dauxois model is presented. We introduce a Monte Carlo technique that allows accurate determination of the dependence of the unzipping forces on unzipping speed and temperature. Our findings agree quantitatively with experimental results for homogeneous DNA, and for λ -phage DNA we reproduce the recently obtained experimental force-temperature phase diagram. Finally, we argue that there may be fundamental differences between *in vivo* and *in vitro* DNA unzipping.

DOI: [10.1103/PhysRevLett.96.248101](https://doi.org/10.1103/PhysRevLett.96.248101)

PACS numbers: 87.15.Aa, 05.90.+m, 64.70.-p

Separation of double-stranded DNA (dsDNA) into single-stranded DNA (ssDNA) is fundamental to DNA replication and other important intracellular processes in living organisms. In equilibrium, DNA will denature when the free energy of the separated ssDNA is less than that of the dsDNA. Because of the larger entropy of the flexible single strand, this can most easily be achieved by increasing the temperature of the sample until the DNA melts, somewhat above body temperature. In living organisms, however, DNA separation is not only thermally driven, but also enzymes and other proteins may force the two strands apart.

Recent advances in single-molecule force spectroscopy and dynamical force spectroscopy (DFS) has made possible the systematic investigation of force-induced separation of dsDNA at room temperature where dsDNA is thermally stable in the absence of an applied force [1]. Although, these studies have significantly enhanced the understanding of the mechanical aspects of DNA replication and transcription *in vivo*, it is also imperative for further developments of technologies, such as polymerase chain reaction and DNA chips, to understand the relation between thermal denaturation and force-induced separation. An initial step in this direction was taken by Danilowicz *et al.* [2] who published an experimentally determined phase diagram for the denaturation temperature as a function of the applied force. This study showed, as theoretically predicted [3], that the force required to unzip the DNA decreases with increasing temperature. However, the applied theoretical framework [2] does not capture all details in the entire temperature range.

The mechanical unzipping of DNA has also been a subject of several theoretical studies, which have often concentrated on macroscopical aspects by investigating thermodynamic equilibrium conditions [4]. Simulating realistic dynamics is unfeasible since the time scales reachable in molecular dynamics simulations are orders of magnitude smaller than in experiments [5]. In this work we present a simple and efficient numerical Monte Carlo (MC) approach, to describe the unzipping process macroscopically as well as semimicroscopically. In particular, we

provide a theoretical underpinning for the experimental force-temperature phase diagram recently published by Danilowicz *et al.* [2].

For this purpose, we use a simple one-dimensional model of DNA proposed by Peyrard-Bishop-Dauxois (PBD) [6]. This model has been demonstrated [7–9] to describe the thermally generated large amplitude local fluctuations (“bubbles”) quite accurately, an aspect which is generally ignored in the thermodynamic models. We will show here that this model successfully describes many of the micromechanical unzipping properties of DNA and provides significant insights into the physical phenomena governing unzipping.

In a typical unzipping experiment, one strand of the DNA molecule is tethered, at the terminating base, to a fixed surface while the other strand is connected through a polymeric linker to a force probe, such as a laser trap or an atomic force microscope (AFM) cantilever. For the sake of simplicity, the force probe is usually treated as a linear spring whose elastic properties can be determined through the system calibration. The force required to keep the molecule extended at a given distance is determined by measuring the deflection of the force probe.

By pulling the molecule apart at a constant speed, the force-extension curves of the system can be obtained. It should be mentioned, however, that the force determined by such techniques cannot be considered a characteristic quantity of the DNA, because it strongly depends on the elasticity of the experimental setup, as well as on the pulling speed. However, by performing the experiment at a wide range of pulling speeds and temperatures, useful information regarding the energy landscape of the DNA unzipping process can be accumulated.

Similarly to this typical experimental setup, we use the model schematically represented in Fig. 1. In this model there is only 1 degree of freedom describing the relative displacement of each base pair (bp) from its equilibrium position. The hydrogen bonds that hold the two strands together are approximated by a Morse potential (dashed lines), while the stacking interaction between successive base pairs is described by nonlinear springs (solid black

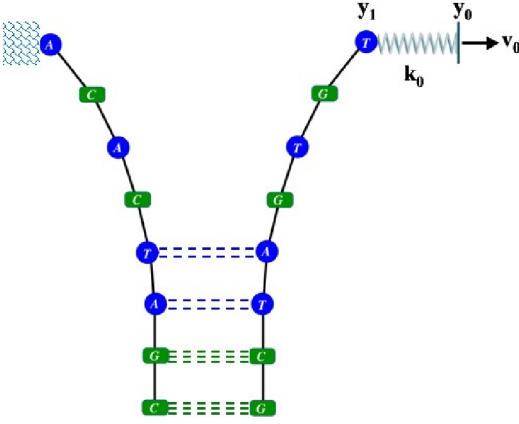


FIG. 1 (color online). A schematic representation of the PBD model in a dynamic force spectroscopy experiment.

line). The model's first base to the left is fixed, while the complementary base is attached to a linear spring (force probe) of stiffness k_0 which moves at a constant speed v_0 . The force probed by the spring is therefore given by Hook's law, $V'_{\text{pull}} = k_0(y_0 - y_1)$, where y_1 and y_0 are the displacements (see Fig. 1) of the first base pair and the spring's opposite end point, respectively. Specifically, the potential energy of the model is

$$V = \sum_n \left[D_n (e^{-a_n y_n} - 1)^2 + \frac{k}{2} (1 + \rho e^{-b(y_n + y_{n-1})}) (y_n - y_{n-1})^2 \right] + V_{\text{pull}}. \quad (1)$$

The PBD model parameters are those determined by Campa and Giansanti [7]. To compute the force applied during the unzipping process, we performed MC simulations: The force probe is moved to the right ($y_0 \rightarrow y_0 + \Delta y$) by the distance Δy , after which N MC steps are performed in order to compute the average displacement, $\langle y_1 \rangle$ of the first base pair. The force will then be given by $F = k_0(y_0 - \langle y_1 \rangle)$. The more MC steps used for sampling, the closer to equilibrium the system is before the next Δy move occurs. Thus, in this MC framework, the pulling speed can be defined as $v_0 = \Delta y/N$. However, it should be emphasized that no direct comparison to the real time can be made as this depends on the detailed implementation of the MC method. Here, the unit of pulling speed is $V = 10^{-4}$ Å/MC steps.

We first investigate the initiation of the unzipping process. In Fig. 2 we present the force-extension curve of a homogeneous 300 bp AT sequence, for different values of the stiffness, k_0 , of the force probe. A significant force barrier at 2 Å extensions is observed for stiff force probes. This barrier gradually decreases with decreasing stiffness, and finally vanishes for $k_0 \lesssim 16$ pN/nm (see inset of Fig. 2). A simple interpretation of this result can be understood by considering the energy landscape of the entire system (DNA and force probe) [10]. Assuming, for simplicity, the unzipping of the first base pair only, for small

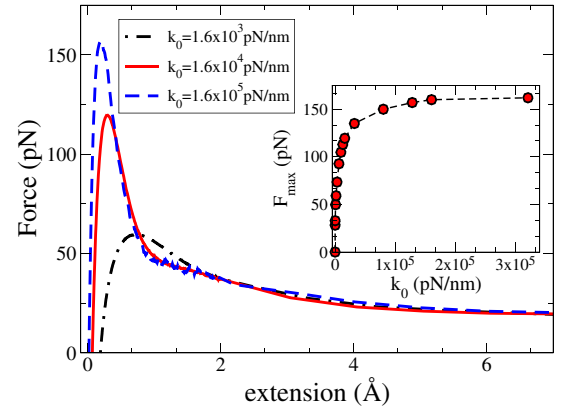


FIG. 2 (color online). Force-extension curves of a 300 bp homogeneous AT sequence for three different values of the probe stiffness k_0 . The inset shows the height of the force barrier, F_{max} , as a function of k_0 . All curves correspond to averaging over 10^3 MC simulations at $T = 300$ K and $v_0 = 0.1$ V.

k_0 , the total energy has two local minima separated by an activation barrier. The first minimum corresponds to the bound state of the base pair and the second to the unbound state. As the force probe moves, the energy landscape is increasingly tilted, and eventually the second minimum becomes the global minimum. Beyond this point the unbound state is the energetically most favorable one. In the simulation (and also in the experiment) only the transition between the bound and unbound state can be observed. States close to the activation barrier are difficult to probe, since the probability decays exponentially with increasing barrier height. For large k_0 , however, the elastic energy of the force sensor becomes dominant and the total energy has only a single minimum such that the unzipping process is continuous with no inaccessible regions. In fact, for very stiff probes ($> 1.6 \times 10^5$ pN/nm) the resulting force accurately represents the derivative of the potential energy with respect to the extension. In the PBD model, the force barrier originates from a combination of the force needed to break the hydrogen bond and the force needed to overcome the entropic barrier of the stacking interaction [11,12]. However, it is important to note that this barrier can be observed only for the unzipping of the first base pairs. When the first base pair is unzipped, the effective probe consisting of the actual force probe and the newly formed single strand becomes very soft and the observation of the force barrier is no longer possible. An experimental indication of the existence of this force barrier can be found in the work of Krautbauer *et al.* [13], where significant force barriers are observed at the initiation of the unzipping process. However, these authors attributed this barrier to interactions with other molecules. The experimental ambiguity of this barrier may be attributed to two factors: First, the experimental apparatus does not have a resolution of 2 Å where the barrier exists. Second, the typical total stiffness of the force probe and the polymeric linkage is very small. The question of whether this force

barrier exists when enzymes bind the DNA molecules obviously depends on how stiff their interaction is. The development of more sophisticated experimental techniques able to accurately probe protein-DNA interaction will shed light on to what extent the binding process is driven by enzymes or thermal fluctuations [8] and supercoiling effects [14]. In what follows, we use $k_0 = 1.6$ pN/nm, corresponding to a value of the force probe stiffness between the lower limit of AFM cantilevers and the upper limit of a typical optical tweezer.

One of the most common questions investigated by DFS experiments is the dependence of the unzipping force on pulling speed. In Fig. 3(a) we present the simulated force-extension curve of a homogeneous AT sequence for three different values of v_0 , at $T = 300$ K. It is seen that faster pulling leads to a higher measured force, in agreement with experiments [15]. However, for small v_0 (~ 0.2 V) the measured force remains practically constant during the simulation, indicating that the system is close to equilibrium. This force corresponds to the experimentally observed unzipping force. During the unzipping of long DNA molecules, a slight increase in the slope of the force-extension curve is also observed, corresponding to the elastic energy of the stretched single strand and force probe system [4]. It should also be noted that, for even slower pulling speeds, the molecule unzipping can take place more easily. This occurs because there is sufficient time for the system to be stochastically driven over the activation barrier at even lower forces [15].

According to our numerical results, presented in Fig. 3(b), the unzipping force of homogeneous AT and

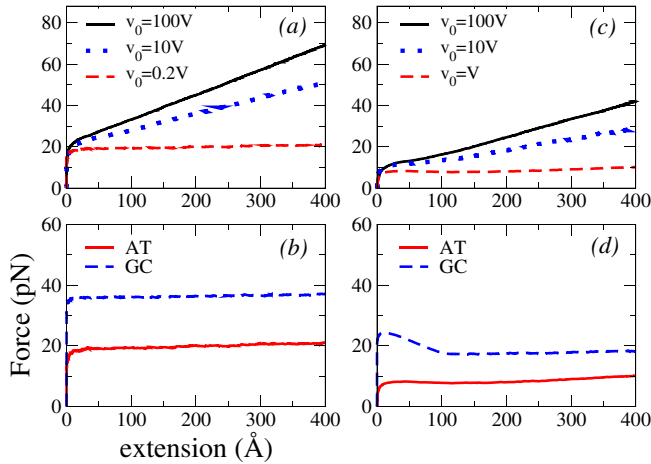


FIG. 3 (color online). Force-extension curves obtained through MC simulations for (a) AT sequence at three different pulling speeds, (b) AT and GC sequences at $v_0 = 0.2$ V and $v_0 = 0.1$ V, respectively. Panels (c) and (d) are the same as (a) and (b) but using the improved description of the single strand stacking interaction (see text for details). In (d) $v_0 = V$ and $v_0 = 0.16$ V for AT and GC sequences, respectively. Each curve is the average over 10^3 independent simulations. In all cases $T = 300$ K, $k_0 = 1.6$ pN/nm, and the number of unzipped base pairs is 300.

GC sequences is 20 and 36 pN, respectively. These values are roughly twice what one would expect from experiments [16,17]. To understand the source of this difference one should recall that the PBD model was originally developed in order to study unforced thermal denaturation. In this context, the choice of only 1 degree of freedom, which describes the relative motion of a base pair, was sufficient. However, this picture does not accurately describe the single-strand dynamics in the kind of experiments we are considering here. During unzipping, two dynamically uncorrelated single strands form, and so at the macroscopic scale of the experiment there is a small possibility for two long single strands to be completely recombined. In the PBD model, the single strands are always dynamically correlated. As a result, in our numerical simulations, the single strands lack entropy, resulting in artificially high unzipping forces.

In reality, j unzipped base pairs produce two single strands each consisting of j bases. The two single strands should be considered as a series of springs with effective stiffness $k/2j$, rather than as in the PBD model where the effective stiffness is k/j . A simple way of imposing this effective entropy increase on the PBD model is to reduce the strength of the stacking interaction, in the single-stranded region, to $k/2$. This requires the introduction of a displacement threshold y_{th} beyond which this transition takes place. The choice of this threshold does not critically affect the result, since our interest lies in describing the behavior at the macroscopic scale of the experiments.

In Figs. 3(c) and 3(d) we present the numerical results obtained from this approach. The resulting rate dependence on the calculated force is exactly the same as in Fig. 3(a), but the unzipping force of homogeneous AT and GC sequences is 9 and 17.5 pN, respectively, values that are in excellent agreement with the existing experimental results [16,17]. It is important to emphasize that *in vivo* unzipping is expected to require more force than the experiments indicate [18]. Existence of the force barrier and the fact that the unzipping experiments probe the locally required force through a long and flexible single-strand linker yields a lower unzipping force. In nature, enzymes apply the force directly to the DNA lacking the flexible linker that significantly lowers the measured force [15]. Indeed, simulations of the unmodified PBD model indicate that an enzyme is required to apply a force of at least 20–36 pN to unzip the DNA molecule. It is clear that our model and numerical approach will be able to shed new light on fundamental processes, such as replication and transcription when applied to interpret single-molecule experimental data obtained for these process [19,20].

With the MC technique and the single-strand modification to the PBD model described earlier, we are now able to determine the force required to unzip DNA at a given temperature, and compare directly with the experimental results of Danilowicz *et al.* [2]. These authors presented the force-temperature phase diagram of the λ -phage DNA at a constant force, compared with the prediction of a simple

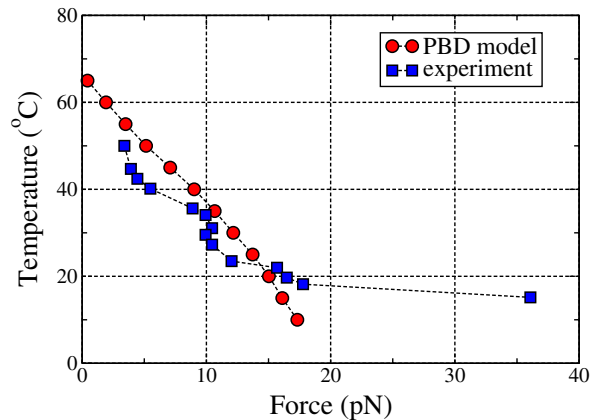


FIG. 4 (color online). Force-temperature phase diagram the λ -phage DNA. Squares correspond to the experimental results of Ref. [2]. Circles represents our numerical results for $k_0 = 1.6$ pN/nm and $\nu_0 = 0.16$ V. Each point is the mean value of the force-extension curve obtained by unzipping the first 300 bp of the molecule for 10^3 different simulations.

thermodynamic model. Figure 4 depicts our results obtained for the unzipping of the first 300 bp of the λ DNA. Since the molecule under investigation is not homogeneous, the unzipping force at each temperature corresponds to the mean value of the force-extension curve. As shown, our results are in very good agreement in the temperature range of 20–50 °C. We notice a difference between our prediction and the experimental results for $T < 20$ °C. This low temperature behavior may be due to a configurational change [2], or to time scales of experimental techniques. Our predictions are more consistent with the experimental results than those of the thermodynamic model. This improvement is most evident at high temperatures, with the thermodynamic model yielding significantly higher forces. This is mainly attributed to the fact that the spontaneous bubble formation for $T > 40$ °C [21] is not described by the thermodynamic model. The bubble formation, which is predicted by the PBD model [9], leads to a considerable reduction of the measured force at those higher temperatures.

In summary we have presented a MC approach to mechanical unzipping of DNA, within the framework of the simple PBD model. Our method allows us to investigate the dependence on unzipping speed and temperature. We demonstrated and analyzed the existence of a force barrier at the initiation of forced unzipping which has generally been ignored in experiments. Further, we found that the single strand, as it extends between the double-stranded molecule and the force probe, causes a decrease in the measured force. We showed that a simple modification of the PBD model is sufficient to appropriately account for this effect and match experimental results. However, we find that the unmodified PBD model reliably captures the local dynamics involved in the unzipping of DNA. With

the described modification, we were able to quantitatively reproduce the experimental force-temperature phase diagram recently obtained for λ -phage DNA. Evidently, the PBD model successfully encompass both the traditional thermal separation and the more recently investigated force-induced DNA unzipping. It therefore offers significant predictive power for *in vivo* situations as well as emerging technologies.

We thank Dr. Steven Koch for informative discussions. Work at Los Alamos is performed under the auspices of the U.S. Department of Energy under Contract No. W-7405-ENG-36.

- [1] R. S. Conroy and C. Danilowicz, *Contemp. Phys.* **45**, 277 (2004).
- [2] C. Danilowicz, Y. Kafri, R. S. Conroy, V. W. Coljee, J. Weeks, and M. Prentiss, *Phys. Rev. Lett.* **93**, 078101 (2004).
- [3] D. R. Nelson, *NATO Science Series II: Mathematics, Physics and Chemistry* **160**, 65 (2004), and references therein.
- [4] U. Bockelmann, Ph. Thomen, B. Essevaz-Roulet, V. Viasnoff, and F. Heslot, *Biophys. J.* **82**, 1537 (2002).
- [5] M. Peyrard, *Europhys. Lett.* **44**, 271 (1998).
- [6] M. Peyrard and A. R. Bishop, *Phys. Rev. Lett.* **62**, 2755 (1989); T. Dauxois, M. Peyrard, and A. R. Bishop, *Phys. Rev. E* **47**, R44 (1993).
- [7] A. Campa and A. Giansanti, *Phys. Rev. E* **58**, 3585 (1998).
- [8] C. H. Choi, G. Kaloskas, K. Ø. Rasmussen, M. Hiromura, A. R. Bishop, and A. Usheva, *Nucleic Acids Res.* **32**, 1584 (2004).
- [9] S. Ares, N. K. Voulgarakis, K. Ø. Rasmussen, and A. R. Bishop, *Phys. Rev. Lett.* **94**, 035504 (2005).
- [10] H. Qian and B. E. Shapiro, *Proteins: Struct., Funct., Genet.* **37**, 576 (1999).
- [11] S. Cocco, R. Monasson, and J. F. Marko, *Phys. Rev. E* **65**, 041907 (2002).
- [12] N. Singh and Y. Singh, *Eur. Phys. J. E* **17**, 7 (2005).
- [13] R. Krautbauer, M. Rief, and H. E. Gaub, *Nano Lett.* **3**, 493 (2003).
- [14] C. J. Benham, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2999 (1993).
- [15] E. Evans and K. Ritchie, *Biophys. J.* **76**, 2439 (1999).
- [16] B. Essevaz-Roulet, U. Bockelmann, and F. Heslot, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 11935 (1997).
- [17] M. Rief, H. Clausen-Schaumann, and H. E. Gaub, *Nat. Struct. Biol.* **6**, 346 (1999).
- [18] S. J. Koch, A. Shundrovsky, B. C. Jantzen, and M. D. Wang, *Biophys. J.* **83**, 1098 (2002).
- [19] Taekjip Ha, Ivan Rasnik, Wei Cheng, Hazen P. Babcock, George H. Gauss, Timothy M. Lohman, and Steven Chu, *Nature (London)* **419**, 638 (2002).
- [20] R. J. Davenport, G. J. L. Wuite, R. Landick, and C. Bustamante, *Science* **287**, 2497 (2000).
- [21] Y. Zeng, A. Montrichok, and G. Zocchi, *Phys. Rev. Lett.* **91**, 148101 (2003).